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Three organophosphorus acid anhydrases have been isolated from E. coli by gel filtration and ion exchange column procedures, and further identified by gel electrophoresis. All three have molecular weights in the 120 000 - 140 000 range. Two of them hydrolyze racemic 1,2,2-trimethylpropylmethylphosphonofluoridate (soman) to completion at a single rate and, in parallel with this, detoxify soman at a comparable rate. The third enzyme appears to show stereoselectivity with respect to the two pairs of isomers of soman in that it hydrolyzes the racemic mixture at a fast and a slow rate, the latter approaching the nonenzymatic rate, and detoxifies soman only at a slower rate. In the past, organophosphorus acid anhydrases from bacterial and mammalian sources have been assayed either as crude sonicates or homogenates, or as cold ethanol precipitated fractions. Major discrepancies among laboratories have probably been due either to the assay of mixtures of varying proportions of these three enzymes depending on the various organs or organisms used as the source, or to the purification of one of the enzymes at the expense of the others. For E. coli, a fourth organophosphorus acid anhydrase is also present but at a considerably lower activity.

Detoxification, DFPase, OPA Anhydrase, Soman, Stereoisomers,  
1,2,2-Trimethylpropylmethylphosphonofluoridate

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## STEREOSELECTIVITY OF SOMAN DETOXICATION BY ORGANOPHOSPHORUS ACID ANHYDRASES FROM *ESCHERICHIA COLI*

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### SUMMARY

Three organophosphorus acid anhydrases have been isolated from *E. coli* by gel filtration and ion exchange column procedures, and further identified by gel electrophoresis. All three have molecular weights in the 120 000–140 000 range. Two of them hydrolyze racemic 1,2,2-trimethylpropylmethylphosphonofluoridate (soman) to completion at a single rate and, in parallel with this, detoxify soman at a comparable rate. The third enzyme appears to show stereoselectivity with respect to the two pairs of isomers of soman in that it hydrolyzes the racemic mixture at a fast and a slow rate, the latter approaching the non-enzymatic rate, and detoxifies soman only at the slower rate. In the past, organophosphorus acid anhydrases from bacterial and mammalian sources have been assayed either as crude sonicates or homogenates, or as cold ethanol precipitated fractions. Major discrepancies among laboratories have probably been due either to the assay of mixtures of varying proportions of these three enzymes depending on the various organs or organisms used as the source, or to the purification of one of the enzymes at the expense of the others. For *E. coli*, a fourth organophosphorus acid anhydrase is also present but at a considerably lower activity.

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**Key words:** Detoxication — DFPase — OPA Anhydrase — Soman — Stereoisomers — 1,2,2-Trimethylpropylmethylphosphonofluoridate

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### INTRODUCTION

Although Mazur [1] did not use the term 'DFPase' to denote an enzyme he had found in mammalian tissues, the name continued in use until a recent interna-

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tional meeting [2] recommended organophosphorus acid anhydrase (OPA anhydrase). Nomenclature aside, for the next decade partial purification involved cold ethanol fractionation without mention whether an ammonium sulfate step had been attempted [1,3-5]. Tests in this laboratory have shown what was probably already known but not published, namely, that the ubiquitous OPA anhydases (Mazur type in our terminology) are ammonium sulfate labile. In contrast, the OPA anhydrase discovered in squid nerve [6] survives 60-65% ammonium sulfate precipitation and subsequent column procedures [7,8]. Other differences between these two enzyme categories — molecular weights, substrate specificities, cation requirements — have been summarized in a recent chapter [9].

The narrowly distributed and sharply defined squid type OPA anhydrase has consistently hydrolyzed and thus detoxified\* all four diastereoisomers of 1,2,2-trimethylpropylmethylphosphonofluoridate (soman), albeit one pair more rapidly than the other [10-12]. The Mazur type OPA anhydases have given conflicting results, ranging from an almost absolute stereospecificity, with no parallel between hydrolysis and detoxication to the opposite [11,13-15]. From time to time we have also seen, but only once reported [16], separable peaks of OPA anhydrase activity from a Mazur type source.

The present report deals entirely with a bacterial source of OPA anhydrase, but the results may provide a broad explanation of the hydrolysis-detoxication anomalies based on the more specific properties of subsets of what has been loosely termed Mazur type OPA anhydases.

## METHODS

### *Enzyme assays*

Use of the fluoride-sensitive electrode for OPA anhydrase determinations has been described repeatedly, and is given in step-by-step detail in a recent chapter [9]. For the 'hydrolysis to completion' experiments (see Figs. 4 and 5) wherein large volumes of enzyme are required, this volume was lyophilized directly in the 10 ml reaction vessel and then reconstituted in the desired volume of buffer fortified with  $Mn^{2+}$ . For the semiquantitative determinations of OPA anhydrase on electrophoresis gels, illustrated in Fig. 6, the gels were sliced into 5 × 50 mm strips (1-2 mm thick), crudely ground in 2 ml buffer in a loose-fitting glass homogenizer, washed into a reaction vessel with another 1.5 ml buffer, and the reaction started by addition of 1.5 ml 0.01 M soman.

Determination of the loss of acetylcholinesterase (AChE)-inhibitory potency (see Table I) has also been described in detail in the recent chapter [9], as well as in earlier publications.

\*For brevity, 'detoxified' and other forms of this word are used with reference to both whole animal toxicity and the inhibition of acetylcholinesterase, these two properties of the compounds under consideration in this paper now linked beyond question.

### Enzyme source

The source of OPA anhydrase with which this paper is concerned is *E. coli*, ATCC25922. Except for growth at 37°C, the medium and conditions were similar to those for the growth of an obligate thermophile (55°C) reported at the First International Meeting on Esterases Hydrolyzing Organophosphorus Compounds held in 1988 [12]. The supernatant from the centrifuged sonicate of the harvested *E. coli* cells was concentrated on a PM-10 (Amicon) membrane, the concentrate was applied to a 2.5 × 90 cm G-150 (Pharmacia) column, and the column was eluted with the sonicate buffer. The fractions identified with individual peaks of activity were pooled, dialyzed, and (except for aliquots retained for further examination) applied to a 1.5 × 30 cm A-50 (Pharmacia) column. The column was eluted with a linear gradient, the limit of which was 20 mM Tris, 0.5 M NaCl. Peak fractions were again dialyzed. All solutions were fortified with  $Mn^{2+}$  at 0.1 mM, and made 1 mM prior to assay.

### RESULTS

Figure 1 shows the elution of protein and OPA anhydrase activity from a G-150 gel filtration resin. The activity peak labelled 'I' was not consistently found, and

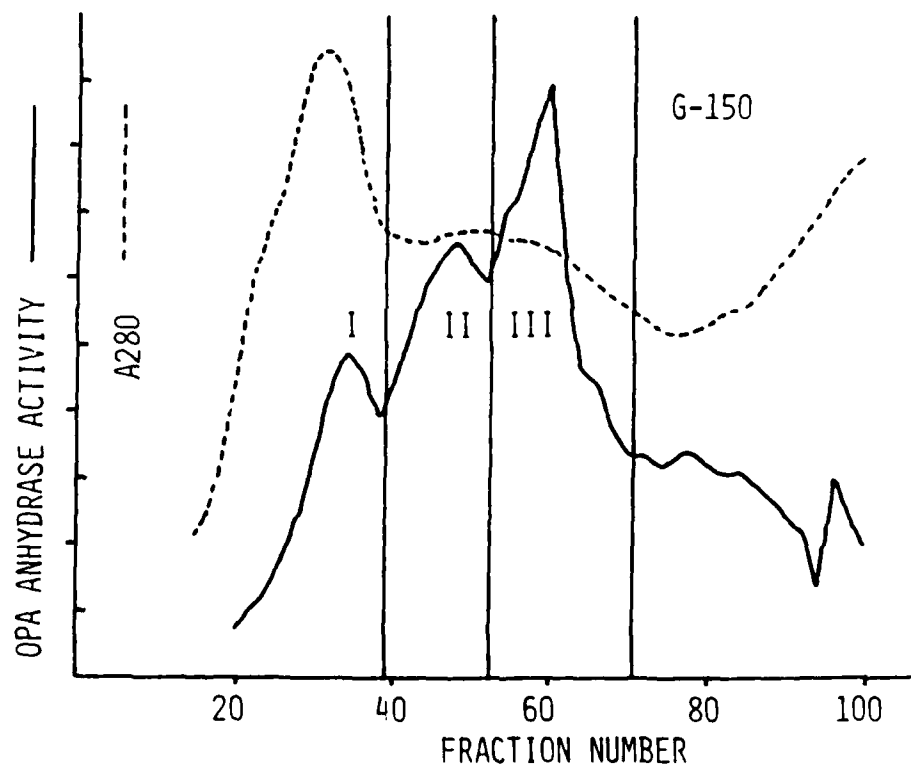


Fig. 1. Elution of the supernatant from sonicated *E. coli* from a gel filtration resin (G-150, Pharmacia). Vertical lines indicate pooled fractions.

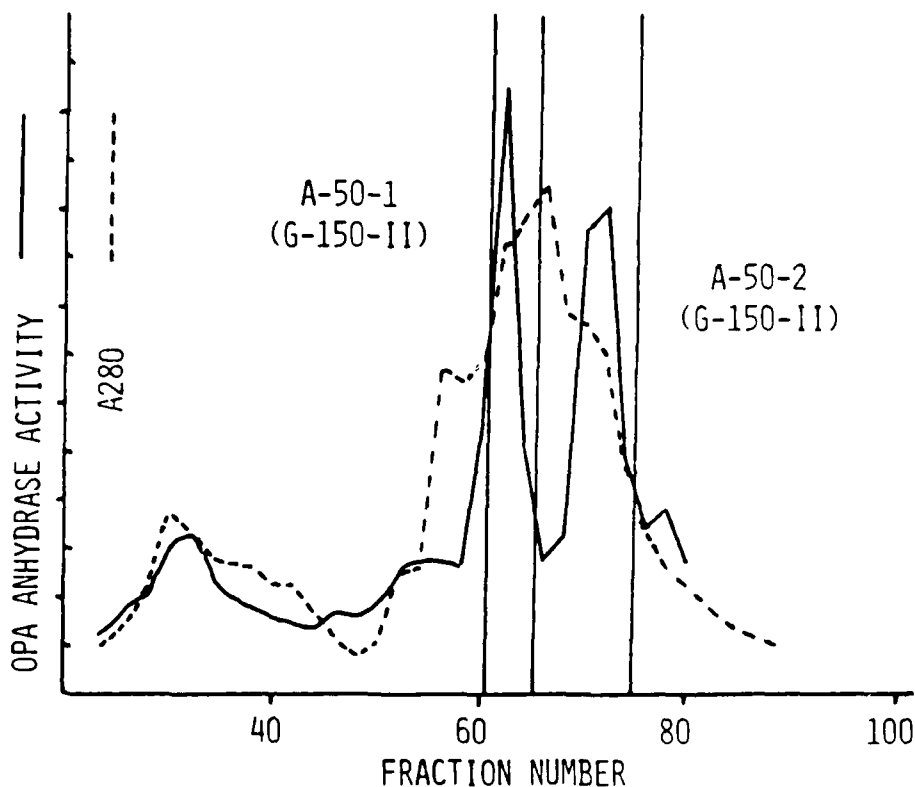


Fig. 2. Elution of fraction G-150-II (see Fig. 1) from an ion exchange resin (A-50, Pharmacia) with a linear gradient, the limit of which is 20 mM Tris, 0.5 M NaCl, pH 7.5.

when found was of considerably lower specific activity than peaks II and III. When G-150-II was eluted from an A-50 anion exchange resin, two sharp peaks of activity were found as shown in Fig. 2; when G-150-III was eluted from A-50, a single major peak was found as shown in Fig. 3.

Small aliquots of several pooled G-150-II, G-150-III, A-50-I (G-150-II), A-50-2 (G-150-II), and A-50 (G-150-III) peaks were tested for their initial rates of soman hydrolysis. Based on this, two larger aliquots of each of these five peaks, estimated to give about the same initial rates, were lyophilized in the reaction vessels that would subsequently be used for 'reactions to completion'. The results of these determinations are shown in Figs. 4 and 5. The second aliquot of each peak was tested for the hydrolysis of soman 'to half completion' and then at that point was tested for the loss of AChE inhibitory potency. These results are given in Table I.

Figure 4 shows that the hydrolysis of soman by G-150-II can be resolved into a fast and a slow reaction. The  $t_{1/2}$  values shown — 10 and 42 min — but not their ratios, depend on the specific activity and size of the aliquot used. A straight line can be fitted, although not as well, giving a  $t_{1/2}$  of about 30 min. A straight line, suggesting a single reaction, seems more appropriate for G-150-III.

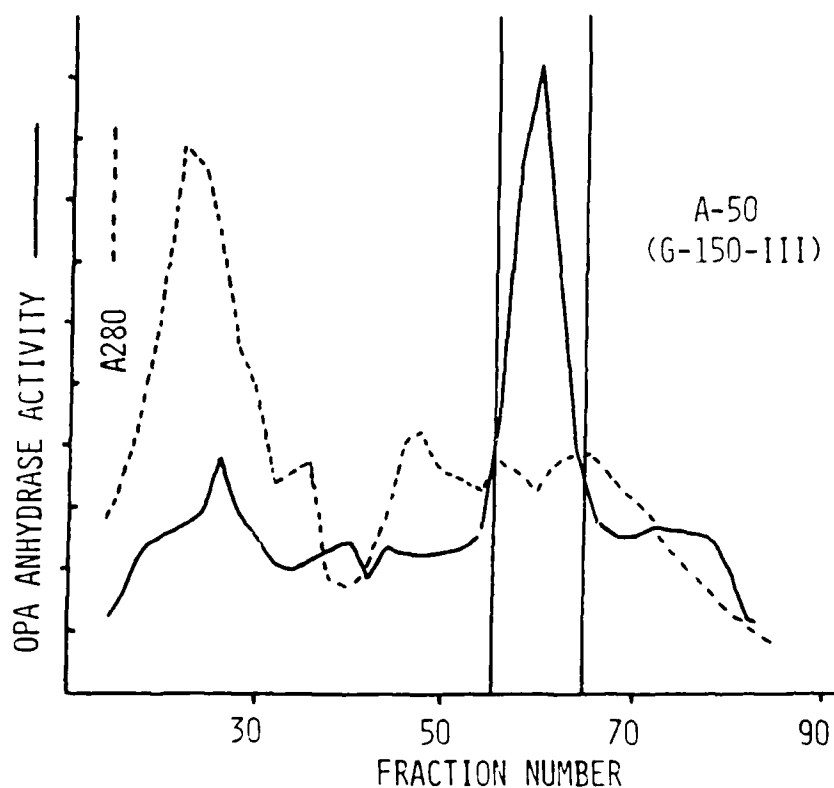


Fig. 3. Elution of fraction G-150-III (see Figure 1) from A-50 resin as in Fig. 2.

Figure 5 shows that the two peaks derived from G-150-II, namely A-50-1 and A-50-2, give single reaction rates for the hydrolysis of soman. In marked contrast, the single A-50 peak derived from G-150-III shows two reaction rates, one about 15 times faster than the other. The obvious discrepancy between these results and those in the previous paragraph will be considered in the Discussion.

Two samples of each of the three A-50 peaks were subjected to gel electrophoresis under relatively non-denaturing conditions. One gel of each fraction was stained with coomassie blue; the second was cut into strips which were assayed for OPA anhydrase with soman as substrate. These semi-quantitative results are presented in Fig. 6.

#### DISCUSSION

The OPA anhydases from *E. coli*, termed collectively Mazur type based mainly on the soman/DFP hydrolysis ratio and  $Mn^{2+}$  requirement, are resolvable into three major Soman-hydrolyzing enzymes. While it might be expected that the G-150-II peak would produce at least one A-50 fraction showing stereoselec-

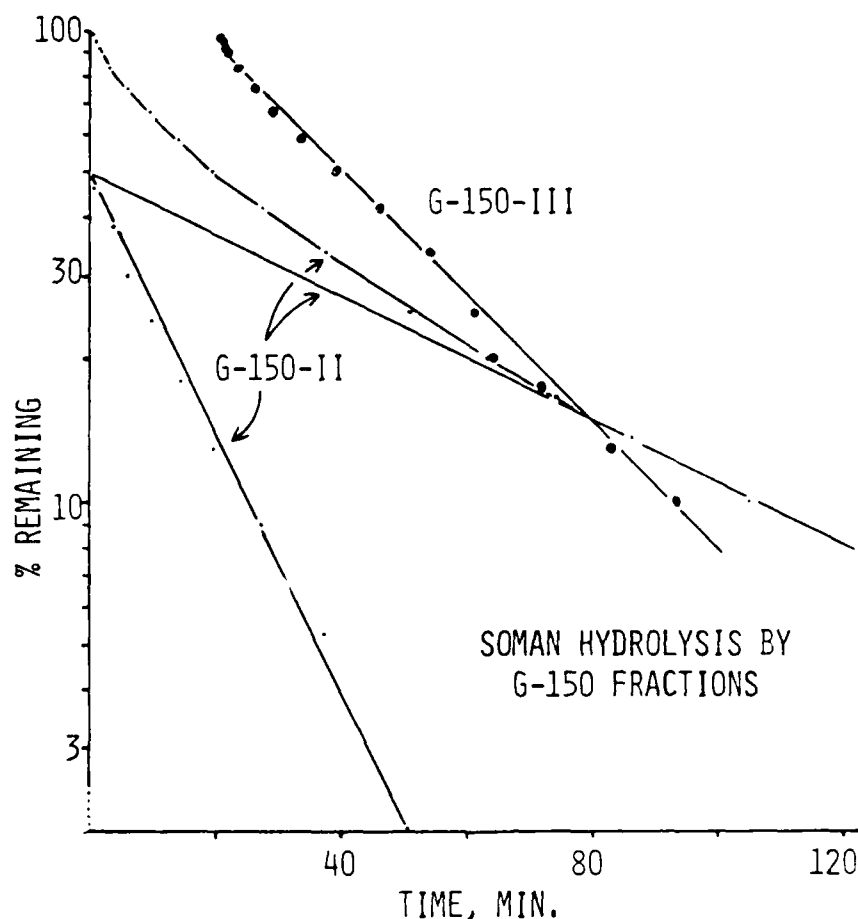


Fig. 4. Hydrolysis of Soman by the two G-150 fractions plotted in a first-order manner. Construction lines are shown for resolving the G-150-II reaction into a fast and a slow component. Hydrolysis of soman by G-150-III is probably a single rate. Reaction lines offset for clarity.

tivity, a consideration of Fig. 5 and Table I shows that this was not found. The G-150-III peak, on the other hand, when further purified on the A-50 resin gave a single peak that hydrolyzes the relatively non-toxic half of the racemic soman about 15 times faster than the toxic half. The discrepancies between the G-150 and the A-50 separations is probably due to the poor resolution of the former. It is clear from Fig. 1 that any cuts of peaks II or III are likely to be cross-contaminated.

*E. coli* as a source of OPA anhydrase appears to contain three, and possibly more, separable P-F splitting enzymes. From repeated tests in our laboratory, and from a consideration of the literature over the past 40 years, it appears that all of these 'non-squid type' OPA anhydases are ammonium sulfate labile. The non-applicability of an ammonium sulfate step has thus eliminated mention of

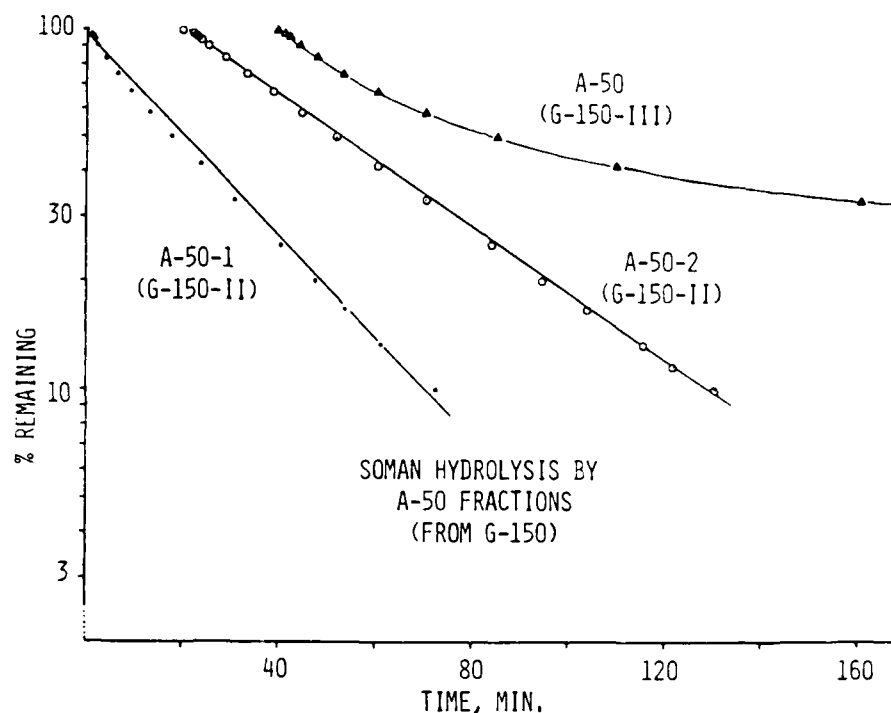


Fig. 5. Hydrolysis of soman by the three A-50 fractions (see Figs. 2 and 3) plotted in a first-order manner. For two of the fractions, single rates appear likely; for A-50(G-150-III), half of the soman is hydrolyzed by a fast reaction and the remainder approaching the non-enzymatic rate. Reaction lines offset for clarity.

this property from the literature. It now appears likely that the cold ethanol fractionation has purified almost exclusively the OPA anhydrase that hydrolyzes only one pair of diastereoisomers of soman [11]. In the present paper this is termed A-50(G-150-III). That this enzyme is different from A-50-1(G-150-II) or

TABLE I

ENZYMATIC DEGRADATION OF SOMAN DETERMINED BY TWO METHODS

Fraction	Hydrolysis by F <sup>-</sup> electrode (%)	Detoxication by AChE inhibition (%)
G-150-II	50	48
G-150-III	50	42
A-50-1(G-150-II)	50	49
A-50-2(G-150-II)	50	50
A-50(G-150-III)	50	18



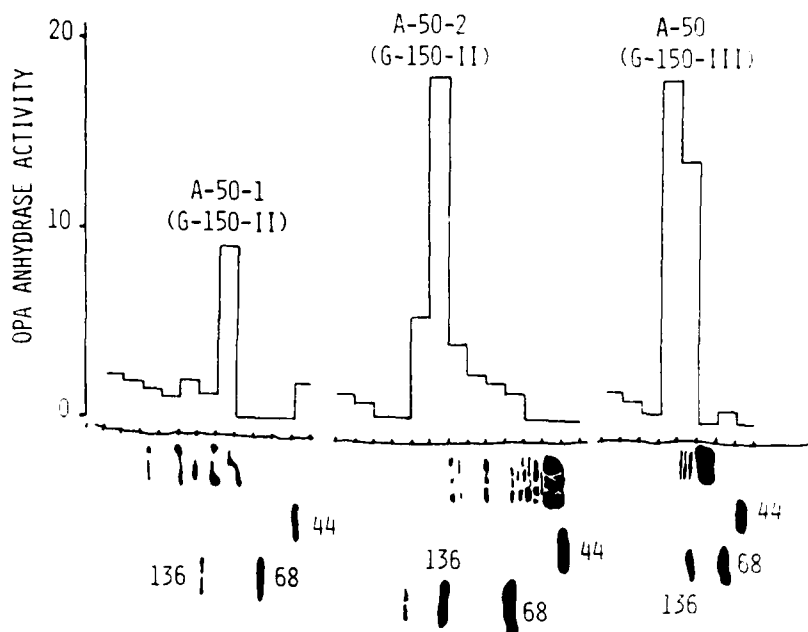


Fig. 6. Gel electrophoresis patterns of the three A-50 fractions and marker proteins (mol. wts. in thousands). The assays for OPA anhydrase are semi-quantitative, so the ordinate scale is relative only. Each segment of this illustration is a composite of photocopies (enhanced) and a graph.

A-50-2(G-150-II) seems clear from Fig. 5. Figure 2 shows that these latter two enzymes are separable on the basis of ionic properties, and Fig. 6 suggests that they may have different molecular weights. It is likely that each of these OPA anhydrases, and the one or two more suggested by the minor peaks in Figs. 1-3, has its own set of properties: soman/DFP hydrolytic ratio, degree of  $Mn^{2+}$  stimulation, mipafox inhibition [17], effects of selected chelators, to list those that are most easily measured. The use of whole organ or organism homogenates has some applicability to toxicity studies, but may suggest by these criteria that there are an almost limitless number of OPA anhydrases. The use of a seeming major OPA anhydrase obtained by a particular purification procedure may produce results not applicable to the functioning of the whole organism. From the data already in the literature [18-20] it seems likely that, with the exception of the cephalopods, the members of most kingdoms and phyla that have been found to exhibit OPA anhydrase activity will contain various proportions of these several OPA anhydrases. The cephalopod enzyme (see ref. 21 for data on individual taxa), i.e., squid type OPA anhydrase, is distinctly different from these several Mazur type OPA anhydrases.

More than a quarter of a century ago, Mounter [18], in his review of the metabolism of organophosphorus anticholinesterase agents, referred to 'several hydrolytic enzymes of overlapping specificities'. The results presented here provide further confirmation of this statement.

## ACKNOWLEDGMENT

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## REFERENCES

- 1 A. Mazur, An enzyme in animal tissue capable of hydrolyzing the phosphorus-fluorine bond of alkyl fluorophosphates, *J. Biol. Chem.*, 164 (1946) 271-289.
- 2 E. Reiner, N.W. Aldridge and F.C.G. Hoskin, *Enzymes Hydrolyzing Organophosphorus Compounds*, Ellis Horwood, Chichester, England, 1989, pp. 246-253.
- 3 L.A. Mounter, C.S. Floyd and A. Chanutin, Dialkyl fluorophosphatase of kidney. I. Purification and properties, *J. Biol. Chem.*, 204 (1953) 221-232.
- 4 J.A. Cohen and M.G.P.J. Warringa, Purification and properties of dialkylfluorophosphatase, *Biochim. Biophys. Acta*, 26 (1957) 29-39.
- 5 K.-B. Augustinsson, Enzymatic hydrolysis of organophosphorus compounds. VII. The stereospecificity of phosphorylphosphatases, *Acta Chem. Scand.*, 11 (1957) 1371-1377.
- 6 F.C.G. Hoskin, P. Rosenberg and M. Brzin, Re-examination of the effect of DFP on electrical and cholinesterase activity of squid giant axon, *Proc. Natl. Acad. Sci. USA*, 55 (1966) 1231-1234.
- 7 F.C.G. Hoskin and R.J. Long, Purification of a DFP hydrolyzing enzyme from squid head ganglion, *Arch. Biochem. Biophys.*, 150 (1972) 548-555.
- 8 J.M. Garden, S.K. Hause, F.C.G. Hoskin and A.H. Roush, Comparison of DFP-hydrolyzing enzyme purified from head ganglion and hepatopancreas of squid (*Loligo pealei*) by means of isoelectric focusing, *Comp. Biochem. Physiol.*, 52C (1975) 95-98.
- 9 F.C.G. Hoskin, An organophosphorus detoxifying enzyme unique to squid, in: D.L. Gilbert, W.J. Adelman, Jr. and J.M. Arnold (Eds.), *Squid as Experimental Animals*, Plenum, New York, 1990, Ch. 21.
- 10 F.C.G. Hoskin and A.H. Roush, Hydrolysis of nerve gas by squid-type diisopropyl phosphorofluoridate hydrolyzing enzyme on agarose resin, *Science*, 215 (1982) 1255-1257.
- 11 G. Chettur, J.J. DeFrank, B.J. Gallo, F.C.G. Hoskin, S. Mainer, F.M. Robbins, K.E. Steinmann and J.E. Walker, Soman hydrolyzing and detoxifying properties of an enzyme from a thermophilic bacterium, *Fundam. Appl. Toxicol.*, 11 (1988) 373-380.
- 12 F.C.G. Hoskin, G. Chettur, S. Mainer, K.E. Steinmann, J.J. DeFrank, B.J. Gallo, F.M. Robbins and J.E. Walker, Soman hydrolysis and detoxication by a thermophilic bacterium, in: E. Reiner, W.N. Aldridge and F.C.G. Hoskin (Eds.), *Enzymes Hydrolyzing Organophosphorus Compounds*, Ellis Horwood, Chichester, England, 1989, Ch. 5.
- 13 H.C. DeBisschop, E.E. VanDriessche, M.L.M. Alberty and J.L. Willems, In vitro detoxification of Soman in human plasma, *Fundam. Appl. Toxicol.*, 6 Part 2, (1985) S175-S179.
- 14 I. Nordgren, G. Lundgren, G. Puu, B. Karlen and B. Holmstedt, Distribution and elimination of the stereoisomers of Soman and their effects on brain acetylcholinesterase, *Fundam. Appl. Toxicol.*, 6 Part 2, (1985) S252-S259.
- 15 J.S. Little, C.A. Broomfield, M.K. Fox-Talbot, L.J. Boucher, B. MacIver and D.E. Lenz, Partial characterization of an enzyme that hydrolyzes sarin, soman, tabun and diisopropyl phosphorofluoridate (DFP), *Biochem. Pharmacol.*, 38 (1989) 23-29.
- 16 F.C.G. Hoskin and K.E. Steinmann, The detoxication of organophosphorus compounds: a tale of two enzymes - or are there more?, in: A.H. El-Sebae (Ed.), *Proceedings of the International Conference on Environmental Hazards of Agrochemicals in Developing Countries*, University of Alexandria Press, Egypt, 1983, Vol. 1, pp. 434-447.
- 17 F.C.G. Hoskin, Inhibition of a Soman- and diisopropyl phosphorofluoridate (DFP)-hydrolyzing enzyme by Mipaflox, *Biochem. Pharmacol.*, 34 (1985) 2069-2072.
- 18 L.A. Mounter, Metabolism of organophosphorus anticholinesterase agents, in: G.B. Koelle (Ed.), *Handbuch der Experimentellen Pharmakologie: Cholinesterases and Anticholinesterase Agents*, Springer-Verlag, Berlin, 1963, Ch. 10.

- 19 F.C.G. Hoskin, M.A. Kirkish and K.E. Steinmann, Two enzymes for the detoxication of organophosphorus compounds — sources, similarities and significance, *Fundam. Appl. Toxicol.*, 4 Part 2, (1984), S165 - S172.
- 20 W.G. Landis and J.J. DeFrank, Enzymatic hydrolysis of toxic organofluorophosphate compounds, in: D. Kamely, A. Chakrabarty and G.S. Osman (Eds.), *Biotechnology and Biodegradation*, Advances in Applied Technology series, Gulf Publishing, Houston, Texas, 1990, Vol. 4, pp. 183 - 201.
- 21 F.C.G. Hoskin and M. Brande, An improved sulfur assay apphed to a problem of isethionate metabolism in squid axon and other nerves, *J. Neurochem.*, 20 (1973) 1317 - 1327.

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